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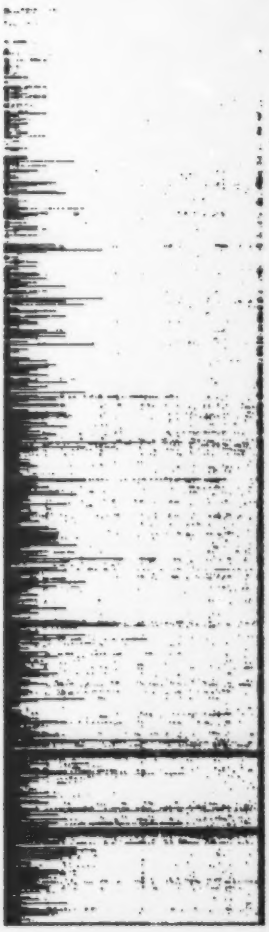
Characterisation of Biological Aerosol with a Fluorescent Aerodynamic Particle Sizer in Outdoor Environment



By:

Jim Ho, M. Spence and P. Hairston

June 1998

WARNING

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Characterisation of Biological Aerosol with a Fluorescent Aerodynamic Particle Sizer in Outdoor Environment

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EXECUTIVE SUMMARY

Biological aerosol measurement in real time is an urgent military requirement that also has many potential non-military applications. Such detection capabilities will be useful in environmental monitoring, for example, in gathering information in perceived hazardous areas like housing developments downwind of sewage treatment plants.

Experience gained from measuring fluorescence signals of single bacterial spores under flow cytometry using UV excitation at 340-360 nm, was applied to concept testing of a prototype instrument, built to do the same for aerosols. This machine was capable of resolving particle size as well as fluorescence intensity of each particle under laboratory and field conditions; it was called the fluorescent aerodynamic particle sizer (FLAPS). A second generation FLAPS (FLAPS2) was designed to be smaller, power efficient and field portable. FLAPS2 was challenged under refereed conditions in blind trials to determine if it could detect biological aerosols. This paper describes practical aspects of measuring biological aerosols when the results must be compared to reference samplers that provide culturable or "live" data. Treatment of particle size and fluorescence information is discussed with respect to FLAPS and reference data fidelity. Finally, an objective method is introduced to evaluate FLAPS data correlation to reference data.

We have shown that sampling of aerosols in the field can be influenced by unexpected environmental conditions. Placement of detectors with respect to referee systems can be crucial to obtaining high fidelity data. There is evidence to suggest that the two generations of FLAPS detectors, built with different laser designs can detect live organisms equally well. Evidence has been provided to indicate particle number analysis by itself is insufficient to characterise a biological cloud. This approach is susceptible to interference by nuisance dust or non-biological particles. By measuring fluorescent emission on UV excitation at 340-360 nm, we have demonstrated that the measured signals closely correlate with live or culturable biological particles. An objective analytical method has been used to define correlation of FLAPS and reference data. We employed standard non-parametric statistical techniques to assist in arriving at the conclusions. This approach facilitated unequivocal comparison between detector performance even though the data came from detector techniques that were fundamentally different. In biological field trial applications, we believe that this is the first time physical measurements are successfully compared to microbiological growth data. We recommend the adoption of this or similarly rigorous statistical methodology for analysis of future biological field trial results.

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ABSTRACT

Biological aerosol measurement in real time is an urgent military requirement that also has many potential non-military applications. Such detection capabilities will be useful in environmental monitoring, for example, in gathering information in perceived hazardous areas like housing developments downwind of sewage treatment plants.

Experience gained from measuring fluorescence signals of single bacterial spores under flow cytometry using UV excitation at 340-360 nm, was applied to concept testing of a prototype instrument, built to do the same for aerosols. This machine was capable of resolving particle size as well as fluorescence intensity of each particle under laboratory and field conditions; it was called the fluorescent aerodynamic particle sizer (FLAPS). A second generation FLAPS (FLAPS2) was designed to be smaller, power efficient and field portable. FLAPS2 was challenged under refereed conditions in blind trials to determine if it could detect biological aerosols. This paper describes practical aspects of measuring biological aerosols when the results must be compared to reference samplers that provide culturable or "live" data. Treatment of particle size and fluorescence information is discussed with respect to FLAPS and reference data fidelity. Finally, an objective method is introduced to evaluate FLAPS data correlation to reference data. The results suggest that there is positive correlation between FLAPS measurements and live biological aerosol particles.

INTRODUCTION

Microorganisms are naturally aerosolised in the atmosphere, often becoming a biological burden to downwind communities. For example, municipal wastewater aeration tanks have been studied and found to produce, by the minute, over 130 viable particles per m^2, about 10% being coliforms (Sawyer et al 1993). Hosing down of dairy processing plants was shown to produce a biological aerosol that remained above ambient concentrations for as long as 40 minutes (Kang and Frank, 1990). Diffusion modeling showed that aerosolised microorganisms could spread infection over a 150 km^2 area among 10 swine herds (Grant et al, 1994). Indoors microbiological air quality in homes and offices has been reported for bacteria and fungi (Lis et al 1997). The bacterial numbers ranged from 200-800 (homes) and 100-500 (offices) viable particles/m^3 while those for fungi were about half as abundant. Fairly similar findings were reported by the US Environment Protection Agency which has recently taken an interest in measuring indoor and outdoor biological aerosol quality (EPA, 1998). Before meaningful health assessments can be made, it is clear that extensive biological aerosol measurements have to be carried out over long periods of time. The job can be executed more efficiently if it can be done in real time without having to resort to using resource intensive microbiological growth assessment methods.

Measurement and detection of biological aerosols in real time has been a difficult task to achieve. Conventional approaches involving growth of biological samples take days to produce results. Nevertheless, as an initial attempt, we developed a system based on a particle sizing instrument that measured some unique size characteristics of biological particles in air to differentiate them from ambient material. Its development was based on work done at DRES (Ho, 1994) in the 1980's using mostly commercial technologies coupled with a knowledge of biological aerosol characteristics from artificial sources. This system employed particle sizing algorithms to distinguish between observed physical characteristics of biological aerosols and background material. The particle sizing instrument estimated particle size by time-of-flight information derived from scattered light. This scheme was based solely on fundamental physics of light scattering and did not measure any intrinsic biological characteristics. Thus dust clouds with similar scatter properties to biological material could cause interferences. The system was deployed as a biological agent detection system and served its purpose at the unique locations of deployment primarily due to predicted well behaved background aerosol characteristics. The utility of this scheme was reflected in its subsequent adoption by other organisations tasked to build biological detectors.

Deploying this detection system at sites that may have highly variable particulate aerosol load may produce unpredictable results, thus compromising reliable detection performance. New methods of detection based on biological principles were clearly needed. In the early 1990's, we explored flow cytometry as a promising approach for characterising biological particles. It was envisioned that this optical method could provide signals that would reveal useful information from a single spore, a model for many biological threat agents. The instrument was designed by Steen (1983) for resolving fluorescence and light scatter from a single bacterium in a liquid stream. This task was previously undoable with conventional flow cytometers

with optics designed for mammalian cells that have much larger cell diameters (Boyle and Steen, 1993).

We showed that flow cytometry provided unequivocal evidence of single spore intrinsic fluorescence when excited by 365 nm light (Ho and Fisher, 1993). At this wavelength, the most probable biological molecule that emits fluorescence is NADH, a key metabolic intermediate of all cellular life systems (Ichinose, 1991). Other important flow cytometry contributions to bacterial studies are summarised by Davey and Kell (1996). Of special interest is their work on describing viability and vitality of single bacterium in a population using flow cytometry (Kaprelyants and Kell, 1992).

From this observation it was clear that measuring fluorescence from individual spores in air could provide a means of enhancing signal-to-noise over background material. Indeed, other workers have used 266-280 nm UV excitation to study characteristics of protein related fluorescence in biological particles (Bronk and Reinisch, 1993). More recently, Chang's group at Yale applied pulsed UV at 266 nm on single bacterial particles with fast electronics that permitted excitation selectivity (Chen et al 1996). In contrast, our approach emphasized the use of 340-360 nm as the excitation wavelength of choice. Numerous reports suggest this wavelength region provides fluorescence information closely associated with biological molecules linked to critical metabolic activity (Kell et al. 1991). Even more meaningful and potentially more definitive was that the fluorescing molecules could be related to living microorganisms (Harrison and Chance, 1970). If proven to be true, this connection would suggest an aerosol detector could potentially distinguish live from dead particles.

In conventional quantitative analytical biochemistry, it has been standard practice to use short UV wavelengths between 260-280 nm to elicit protein fluorescence as a way for concentration estimation (Groves et al. 1968). However, in the biological detection context, fluorescence excitation at this wavelength is not specific to living organisms, as recently acknowledged by Pinnick et al. (1998). In addition there is suggestion that other molecules such as organic pollutants in air interact with ozone to form particles that fluoresce broadly at 300-800 nm when excited with short wavelength UV (Day and Lee, 1983). Employing 340-365 nm as the selective NADH excitation wavelength and measuring the fluorescence at 400-550 nm would provide the sought after selectivity for living organisms required by a reliable biological agent detector.

We have reported on the first generation fluorescence aerodynamic particle sizer (FLAPS1) that demonstrated single biological spore particles in an air stream could be detected (Hairston et al. 1997). It was effective in field trial detection of biological aerosol where no false alarms were encountered (Boulet et al. 1996). Background biological aerosol characteristics measurement using the FLAPS has been described (Ho and Spence, 1998). This paper describes performance of FLAPS2, a field portable instrument optimised for rapid biological aerosol detection.

METHODS AND MATERIAL

Fluorescent Aerodynamic Particle Sizer (FLAPS).

Under contract to DRES, TSI (St. Paul, MN 555164-0394) built FLAPS1 using a HeCd CW laser coupled to their existing APS platform and DRES specifications for fluorescence detection. The success of this project led to a second contract to construct FLAPS2 based on their APS II platform and more recent DRES specifications for fluorescence detection. This instrument is now commercialised by TSI as model 3312a and for their marketing purposes is called "Ultraviolet Aerodynamic Particle Sizer" (UV-APS). By itself, UV-APS produces raw data without data analysis or alarming capabilities.

Proprietary display and alarming software was developed by DRES with Dycor (Edmonton, Alberta) as programming contractor. The original code has been in development since the mid 80's when the APS was used in DRES field biological detection. It evolved into a functional tool, used successfully during JFT1 (Ho et al. 95). Further development work brought the software up to date and it presently is used to control the FLAPS. The name "FLAPS" describes a functional package consisting of the fluorescence enabled APS hardware and DRES software combination.

The FLAPS2 configuration deployed during Joint Field Trial III (JFT3) was equipped with a pulsed UV light source ("StableLight" model S349-30Q, Uniphase, 163 Baypointe Parkway, San Jose, CA 95134, 800-644-8674). It was powered by a diode-pumped Nd:YLF laser frequency tripled to produce 349 nm. Energy per pulse was rated at 30 μJ running at pulse width of about 5 to 8 nsec.

As the pulse energy was higher than optimal, in both configurations, an optical density 1 filter was used to pass only 10% of the laser output to the measurement chamber. This resulted in lower background noise and better signal-to-noise characteristics. The laser was triggered when a particle was detected with a minimum time between triggers of 200 μsec.

To compensate for different flight times due to particle size, a "skeet shooting" look-up table was used to synchronise laser trigger times. In addition, by issuing firmware commands, selective firing could be exercised to excite only particles of desired size characteristics. This feature not only offered a "smart" technique for interrogating the biological characteristics of a single particle, it also avoided unnecessary firing of the laser at background material (<1 μm size).

During real time sampling, the instrument presents size and fluorescence intensity information for each particle. Data derived from a sampling period, typically 3 seconds, can be reduced to a fractional number (gated % fluorescent); this represents the percent of particles that exhibited fluorescent signal above a preset size and intensity threshold. A threshold level can be determined for each environmental condition by observing the prevailing background conditions. An alternate method to present fluorescence data is to derive a "signal-to-noise" ratio. This is done but comparing the mean of background gated % fluorescent with new "unknown" or "biological" data.

As a way to enrich the population of background fluorescent particles for better statistical counting, a prototype aerosol concentrator (model XMX, SCP Engineering, St. Paul, MN) was used as a front end to the FLAPS2 intake. This was a

modified version of the original XM2 used for concentrating biological aerosol, as reported previously (Brenner et al 1988). Our smaller version, designed for optimal size, weight and power consumption operates at 400 liter/min concentrating to 1 liter/min delivered to the FLAPS intake. Improved particle throughput provided by this setup combination facilitated a rapid sampling time of 3 seconds. In addition to this, a 1 second penalty is incurred for computational and data handling overhead.

Field trial protocols.

Field biological aerosol dissemination (*Bacillus subtilis* var niger, BG) and reference sample collection were services provided by Dugway Proving Grounds. Biological aerosol or tracer smoke dissemination took place about 800 m upwind of the detection grid. Andersen and Mohr (1996) described the procedures in detail and only brief comments will be stated here where warranted.

Live particles were impacted onto 15 cm nutrient agar plates rotating at 1 revolution/min using modified slit samplers (model STA 203, New Brunswick Scientific, Edison, NJ 08818-4005). The samplers ran at 28.3 liter/. Each array of 10 samplers per station was located on an elevated platform, set longitudinally adjacent to the FLAPS. This distance was dictated by the width of the array of machines (30 cm each). Air intake height for the reference samplers was about 1 meter higher than that of the FLAPS. Most trials took place with the wind from a south to north direction.

On passage of a test cloud, the reference samplers were sequentially switched on manually by the trial operators. Exact timing for cloud passage was determined by a tracer gas (propylene) that was disseminated simultaneously with the biological aerosol. A series of gas detectors provided real time information to determine the optimal moment for triggering the slit samplers. Precise trigger was critical for effective operation of the reference system. There was a maximum usable collection time of 10 minute and generally, the operators hit the on switch fairly close to biological cloud passage. During this time, biological particles, if present in the aerosol, were impacted on to the surface of a nutrient agar plate. After over night incubation, live particles were counted as bacterial colonies by means of a flat bed scanner driven by custom software developed jointly by DRES, DPG and Spiral Biotech (model "STAR" version 1.5, Spiral Biotech, Bethesda, MD 20814). Reference data, expressed as agent containing particles per liter of air (ACPLA) were provided by Dugway after the trials. In this report, fluorescent data were compared to reference data to illustrate timing relationships whenever biological hits occurred.

Statistical analysis and Data Transform.

In wanting to know if two variables are interdependent or vary together, the common approach is to perform a correlation analysis (Sokal and Rohlf, 1981). A typical assumption is that the two variables are both effects of a common cause. Translated to our present problem, we wish to estimate the degree to which FLAPS measurements vary together with live reference data and the common cause is the presence of live biological aerosols. Before applying this test, there is one precaution to note-- the data must be tested for distribution normality. Non-normally distributed data like FLAPS measurements and slit sampler data (shown in results) require non-parametric methods. Spearman's non-parametric correlation coefficient was

determined by SigmaStat version 2 (SPSS Inc. 444 N. Michigan Avenue, Chicago, Illinois, 60611) as described by Sokal and Rohlf (1981). This test requires 2 data sets of equal size and timing intervals. However, raw slit sampler and FLAPS data sets derived from two fundamentally different measurement approaches, have different timing intervals (2 and 4 sec. respectively). As a consequence, they are structurally dissimilar and unsuitable for analysis.

To overcome this discrepancy, each data set consisting of time and the variable was transformed by first converting the time format into decimal time (e.g. 12:00:00 to 0.5) using the "timevalue" function in Excel (Microsoft Office 97, Microsoft Inc., Redmond, Washington 98052-6399 U.S.A). Then x and y data columns were imported into TableCurve 2D (version 4, SPSS Inc. 444 N. Michigan Avenue, Chicago, Illinois, 60611) where non-parametric "local regression" estimation was done using Gaussian weighting. Three other non-parametric methods were also used to determine the optimal approach (local regression with Loess weighting; spline estimation with non-uniform rational B-spline [NURBS] weighting and smoothing spline estimation with fast fourier transform [FFT] and NURBS weighting). For generating new interpolated data sets, increment decimal time value of 0.000023148 or about 5 sec. was used along with specified beginning and end times. After performing the conversion procedure for both FLAPS and reference data, just the y portions of each set were grouped, constituting the variable pair suitable for Spearman's test.

RESULTS AND DISCUSSIONS

Location of samplers

Existing open air measuring devices recommended for sampling biological aerosols are essentially point detectors (Chatigny et al 1989). When attempting to compare performance of two or more samplers in an environment of unpredictable wind characteristics, it is important to co-locate these devices as closely as possible to avoid sampling errors. Significant errors can occur if two samplers have air intakes of different heights relative to the ground as illustrated in figure 1.

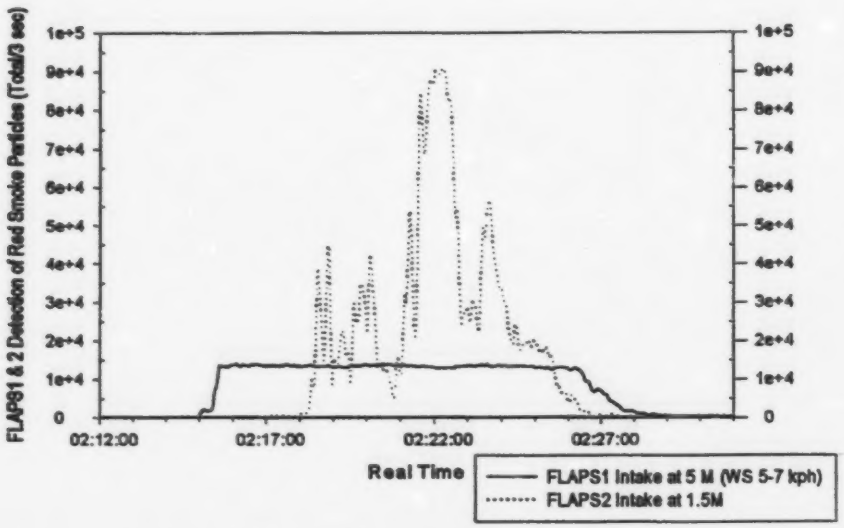

Figure 1. Demonstration of Air Stratification Effects on Particle Distribution Note Detection Hit Time Disparity of 3 Minutes

In this experiment, optically visible red smoke was disseminated to provide a test for determining if interfering aerosols would degrade detector performance. One advantage of using red smoke was that the experimenters could actually see the aerosol passage through the test site. At the time of day shown in figure 1, there was an apparent temperature inversion creating differential air layers at various heights. As a result, the aerosol particles could be observed to move horizontally downwind as a layer at a distance of several meters above ground level. A distinct clear air/aerosol interface was clearly visible, with the particle-laden layer on top. For about 3 minutes, the air below this interface remained clear. This unique observation was confirmed by analysing particle data collected by two FLAPS instruments with air intakes set at two different heights as indicated in this figure. On this occasion, particle numbers were plotted versus time to illustrate the effects of environmental conditions on measurement parameters. Also, there was no detectable fluorescence from red smoke particles. The important lesson to learn here is that due to unusual

environmental conditions, particle detection times appear to vary significantly if aerosol samples were extracted at different heights.

Apart from detecting the smoke at different times, the lower instrument also measured concentrations at almost an order of magnitude higher than the second instrument at around 02:22:00. It is possible that this difference was partially due to complex uneven mixing or interaction of the stratified aerosol layers. It is clear that from this observation, much remains to be learned from open air aerosol measurements under unique kinds of environmental conditions.

This data also provides an extra dimension of awareness to those whose interest is in modeling of aerosol transport. A fairly common practice in aerosol modeling is the expedient use of simplistic environmental assumptions that differ from reality. Grave errors in computational predictions may occur if the lessons shown here are not carefully exploited.

As a result of these observations, we made great efforts at co-locating FLAPS and the reference samplers. This was not as practical as we would have liked as the slit samplers occupied a platform of about 3 meters in width. The closest possible location FLAPS could be positioned was about 1 meter to the right of the platform. Nevertheless, having defined the limitations, we were glad to note that our bioaerosol measurements closely matched the aerosol dynamics of the references samplers in almost all cases.

Fluorescence Signal to Noise

The prototype instrument (FLAPS1) employed a continuous wave HeCd laser as excitation source (Hairston et al 97). After successfully demonstrating the working concept, we set out to reduce its size, weight and power requirements. Hardware constraints dictated that FLAPS2 had to be designed around a pulsed laser resulting in a package that could be housed in a one meter cube enclosure. However, due to this change in laser illumination, it was of interest to demonstrate that the two instruments had comparable performance. Figure 2 shows an experiment where the two instruments were presented with an aerosol cloud in the field under ideal environmental conditions, unlike that encountered in figure 1. In this example, the signal-to-noise ratio was plotted for each showing that the instruments detected the cloud in almost identical fashion. Both instruments detected the leading edge of the cloud at about 21:38:00 where S/N registered about 2. To illustrate a concomitant presence of biological aerosol containing culturable particles, figure 2 also shows the scatter plot for live particles (ACPLA) collected by slit samplers. There is evidence to suggest that in this example, both FLAPS began to register S/N of 3 in the presence of about 10 ACPLA.

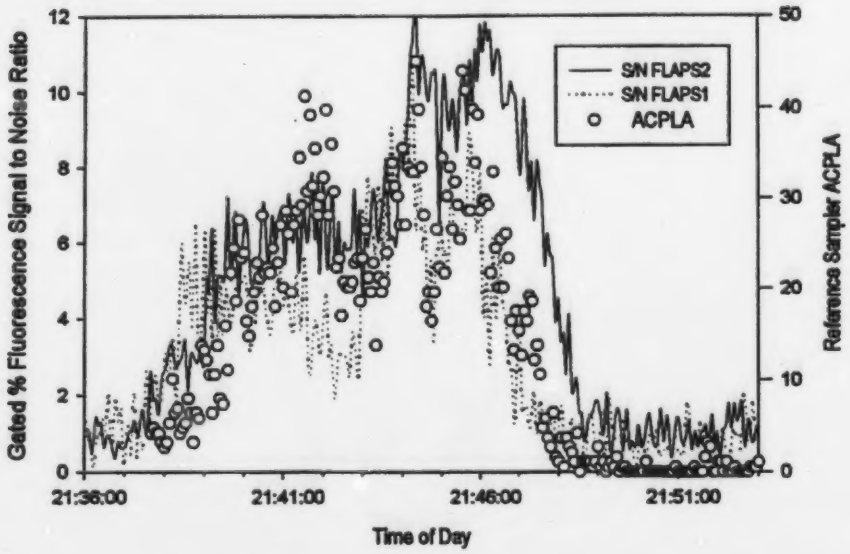

Figure 2. Signal-to-noise Ratio Analysis
Comparison of FLAPS1 and 2 Performance

Five minutes after the initial event, a higher concentration cloud of about 20 ACPLA caused both detectors to register S/N of about 5 to 6. In the next 5 minutes there was apparently a bimodal cloud that was resolved by both instruments as having a "dip" in S/N levels at around 21:45:00. Interestingly, the reference samplers concomitantly detected this event. By 21:49:00, both detectors reported background levels after exhibiting a steeply declining slope from a peak S/N of about 10 to 11. This was equivalent to about 40 ACPLA. Again this event was confirmed by referee data.

The time course data in figure 2 appear to indicate that fluorescent signals from low as well as high biological aerosol concentrations can be unambiguously resolved. The data suggest that the instrument has a useful dynamic range of at least from 5 to 50 ACPLA. We speculate the quick drop in signal levels after passage of a cloud as a noteworthy FLAPS design feature. It is possible that the APS sheath flow may have played a role in contributing to good aerosol clearance characteristics after passage of a fairly heavy biological cloud. In contrast, there are other optical designs that emphasize large surface area exposure of particles in the optics (Jeys et al, 1997) and thus may account for their difficulties with slow clearance of residual material long after the passage of the aerosol cloud.

Particle number measurement versus fluorescence measurement.

One disadvantage in using particle light scatter signals for detection triggering is the presence of ambient non-biological particles that may cause errors. To reduce the potential for false positive detection events, a detector should be designed

to measure real biological entities rather than particle based "noise". Figure 3 illustrates the benefit of using fluorescence based measurements as compared to those base solely on light scatter. It is shown that of the 3 particle based events (01:12:30, 01:22:00 and 01:32:00), only the middle one corresponded to fluorescence and by inference, biological content.

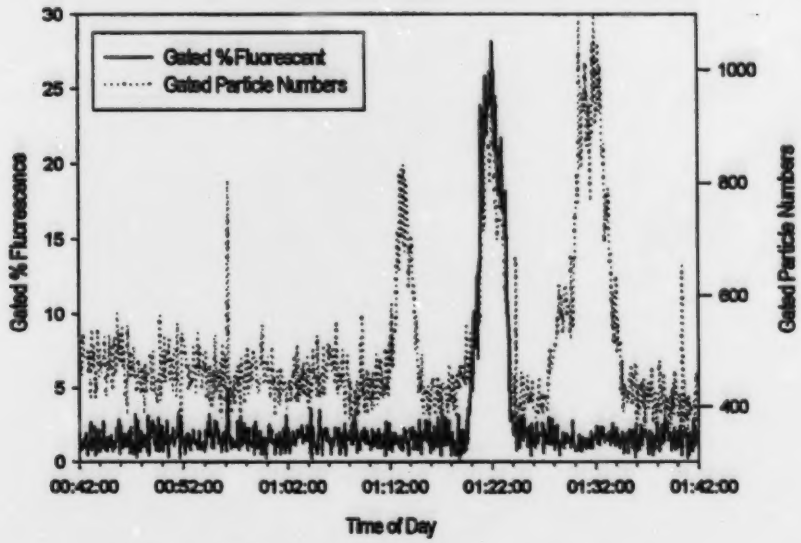

Figure 3. Fluorescent vs Particle Numbers Analysis

When this data set was compared to reference biological data (figure 4), it can be seen that there was a close to perfect temporal match. Note that the time of day scale has been expanded to focus in on the details. It can be seen that the reference data set began at 01:20:49 whereas FLAPS2 indicated the presence of fluorescent signals at about 01:20:00, an apparent discrepancy. However, as was mentioned earlier, the reference samplers were manually activated and occasionally the starting cue could be delayed due to timing errors. In this case, a lag of about 49 seconds resulted in the reference samplers capturing the aerosol cloud well after the initial rising slope. Another reference data anomaly can be seen at 01:24:45 where there was an apparent spike registering 22 ACPLA. This data was not confirmed by any of the 4 other reference sampling locations, suggesting that it might be an anomaly rather than a real event that was not detected by FLAPS.

The rest of the reference data set gave a remarkable visual match with respect to FLAPS2 fluorescent data. From a subjective interpretation viewpoint, the coincidence of these two data sets would lead us to speculate that the two measurement systems were detecting the same phenomenon. It is possible to be convincing using this graphical approach when the data sets show little to no time based

discrepancies. However, there were instances where imperfect matches existed (figure 6), and so a more objective analytical approach was required to assess detector performance.

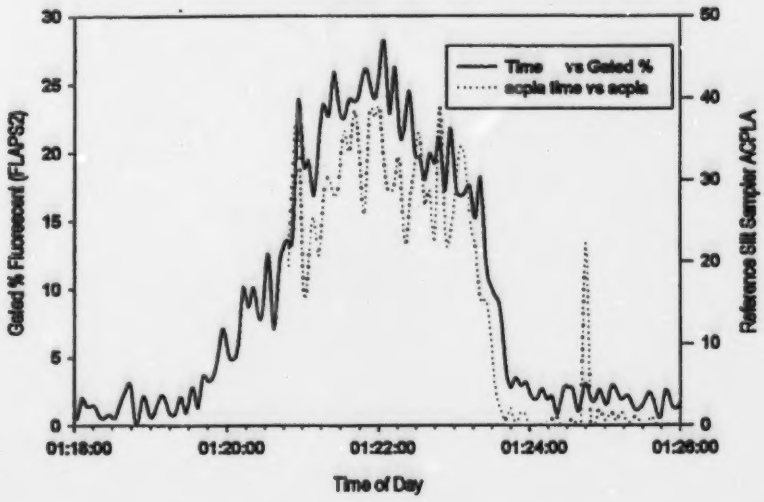

Figure 4. Comparing Fluorescent Data and Biological Reference Data

Although test aerosol dissemination was a continual process, occasionally, most likely due to wind and ground related effects, two distinct biological clouds could be detected. Figure 5 shows an example of two successive hit events. Between these two hits, just before 17:06:00 and only lasting for a few sampling intervals, no live particles were measured by the references samplers. This coincided with a similar interval where FLAPS also registered only background gated % fluorescent levels. This is a good illustration of the resolving power of the FLAPS optical system. It can be seen that the clean air period between the two clouds was clearly defined as less than 10-15 seconds. Yet immediately after that the instrument responded to the second cloud with no obvious lag.

Unlike what happened at the initial aerosol hit point shown in figure 4, here in figure 5 the reference aerosol capture was perfectly executed. This is depicted in a series of clear background data sets representing clean air at 17:01:00. Half a minute later, in the presence of 3 to 5 ACPLA biological aerosol, FLAPS detection was positively registered. Similarly, the rise and fall of aerosol content throughout the two hits were optically tracked with high fidelity.

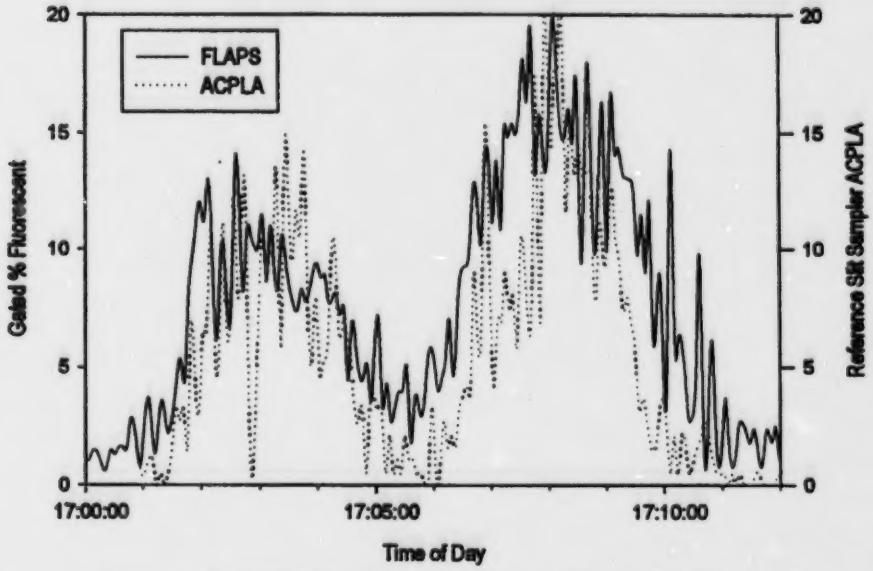

Figure 5. Optically Resolving Two Distinct Biological Clouds

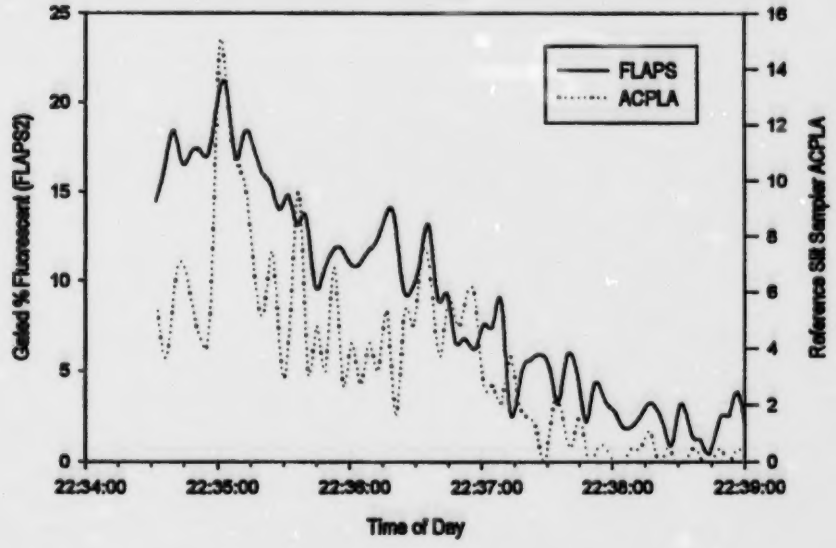

Figure 6. Example of Imperfectly Matched Data Sets

Background aerosol clouds normally consist of a constant proportion of fluorescent particles mixed in with other particles of non-biological origin. However, under high background conditions gated % fluorescent values may become distorted when compared with reference data. This can be especially critical if a high background level of non-fluorescent particles masks a detection event involving a low level of fluorescent particles. Fortunately, this condition has been encountered only rarely but figure 6 shows an instance where slight to moderate distortion occurred. In this example, it is visually less obvious that the two raw data sets describe the same aerosol event. Thus it becomes necessary to employ statistical tools to help evaluate such "difficult" data sets.

Statistical approaches.

Some basic descriptive statistical analysis was done to the particle data sets to determine the correct approaches to take with respect to correlation testing procedures. Figure 7 shows typical example of a test outcome, in this case, using total fluorescent particle data for illustration. With a skewness coefficient of 1.12, the data set was not normally distributed (normal = 0). This is not surprising; the observation that biological data are not normally distributed has been commonly reported before (Tillett and Carpenter, 1991). Similar observations were obtained with the other data sets. For this reason, non-parametric local regression methods were selected for interpolation estimations. As mentioned previously, we needed matched data sets to perform comparison tests and the first step was to select the best transform method. However, since there are a variety of techniques to chose from, a survey study was required to determine the best data transform model.

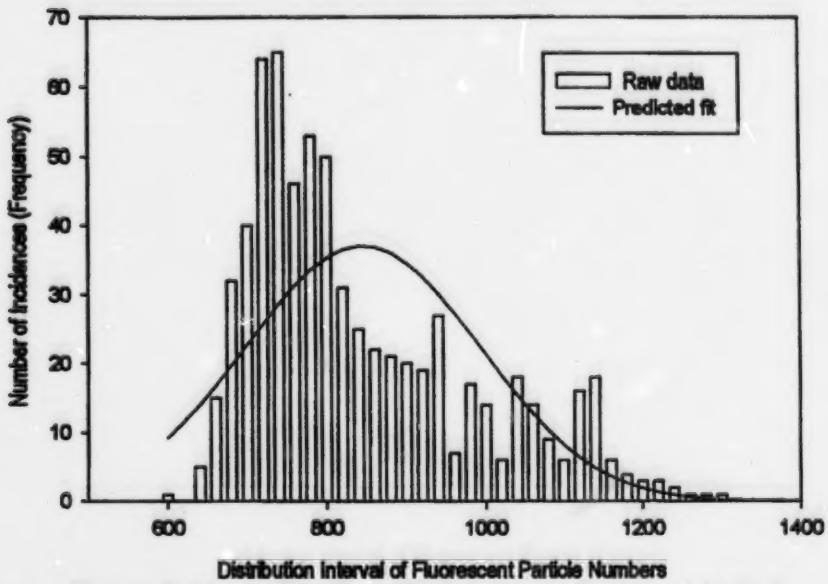

Figure 7. Normality Test (Kolmogorov-Smirnov) Failed
Skewness = 1.12 (normal = 0)
K-S Distance = 0.145 P = <0.001
Tested with total fluorescence data set

Figure 8 shows the results of subjecting raw gated fluorescent particle data to local regression treatment (with gaussian weighting). The new predicted or transformed data compared very well with the original, as indicated by an excellent regression coefficient (r^2 = 0.99). Other methods of regression transform also yielded good coefficients (data not shown). When this transformed data set was compared with the transformed reference data using the non-parametric Spearman's test (Cleveland, 1993), a correlation coefficient of 0.813 was obtained. This high value can be interpreted to mean the two measured parameters are positively correlated. In plain terms, for this data set, the FLAPS measurement can be considered statistically equivalent to the live biological data. To take this inference one step further, it implies what the FLAPS measured was indeed that of the live biological aerosol.

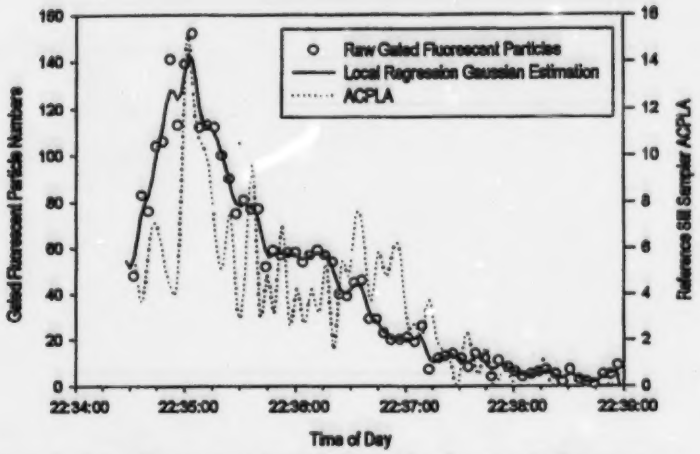

Figure 8. Gated Fluorescent Particle Data Non-Parametric Estimation
Local Regression & Gaussian Weighting $r^2 = 0.99$
Spearman's Coefficient for Gated Fluorescent Numbers vs ACPLA = 0.813

Expanding on what has been shown in figure 8, we proceeded to repeat this treatment on other data sets from the same experiment. The resultant matrix of Spearman's coefficient values is summarised in table 1. The obvious observation is that in all treatments, gated % fluorescent data gave the poorest results. Again we emphasize that since this value is derived from prior numeric manipulation, (it is a fractional ratio number derived from dividing gated fluorescent numbers by gated particle numbers) the lower coefficients obtain are not surprising. Its utility can be appreciated in that it gives an objective evaluation to the same data shown in figure 6 where visual inspection for a fit is difficult to make. A coefficient value of 0.646 (table 1, column 2, last row) can be considered as mildly positively correlated. Its usefulness is apparent when the detectors are used in an environment with wildly fluctuating ambient non-biological content.

By inspecting table 1, local regression with Loess weighting may appear to give better coefficient results. But the numbers did not stand up to statistical (difference) testing, most probably due to insufficient data items. So for practical purposes, any of the four estimation methods will work well. Similarly, gated fluorescent counts may appear to give better test data but the advantage is marginal.

To recap, we have submitted biological aerosol data to rigorous statistical analysis and learned that non-parametric techniques are most appropriate. Accordingly, Spearman's correlation coefficient method was used to determine if FLAPS measurements were a function of live biological aerosol clouds that hit the samplers. The results certainly suggest positive correlation, regardless of which method of data transform was used.

Table 1. Summary of Spearman's correlation with referenced to ACPLA data tested with different estimation methods.

	Local Regression Gaussian	Local Regression Loess	Spline Estimation NURBS	Smooth Spline Estimation FFT & NURBS
Total Particle Count	0.806	0.8822	0.812	0.798
Gated Particle Count	0.813	0.830	0.819	0.803
Total Fluorescent Count	0.806	0.817	0.774	0.800
Gated Fluorescent Count	0.813	0.831	0.821	0.803
Gated % Fluorescent	0.646	0.640	0.655	0.623

Table 1 indicates that under ideal test conditions, it is possible to positively correlate the measured particle parameters with the presence of viable biological aerosol. Although we did not have sufficient replicates to statistically conclude that local regression with Loess weighting was better than the other transform methods, it became our favorite. If we have to make a recommendation as a guide for other workers to treat their biological data, then the following would be fairly representative:

1. test raw data sets for normality distribution
2. use a data transform method to obtain time base compatibility between optical data and biological data
3. if data sets fail normality test, use Spearman's method to measure correlation
4. derive conclusions from positively correlated observations

By following these steps, we would have a uniform approach to objectively make sense of the optical measurements. This is especially crucial when there are numerous optical parameters that can be derive from a single particle. For example, the FLAPS instrument can also measure side scatter. At present, we do not have the transmission speed to cope with another data stream. But soon enough, this bandwidth hurdle will be solved, then it will be relevant to ask if side scatter characteristics can be correlated with biological aerosols.

CONCLUSIONS

1. We have shown that FLAPS works and that it detects living organisms in aerosol; that it is sensitive and unaffected by varying background particle concentrations.
2. We have shown that sampling of aerosols in the field can be influenced by unexpected environmental conditions. Placement of detectors with respect to referee systems can be crucial to obtaining high fidelity data.
3. There is evidence to suggest that the two generations of FLAPS detectors, built with different laser designs, have similar performance characteristics.
4. Evidence has been provided to indicate particle number analysis by itself is insufficient to characterise a biological cloud because it is susceptible to interference by nuisance dust or non-biological particles.
5. By measuring fluorescent emission on UV excitation at 340-360 nm, we have demonstrated that the measured signals closely correlate with live or culturable biological particles.
6. Statistical analyses can be used to enhance raw data sets for improved performance in difficult aerosol conditions.

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Biological aerosol measurement in real time is an urgent military requirement that also has many potential non-military applications. Such detection capabilities will be useful in environmental monitoring, for example, in gathering information in perceived hazardous areas like housing developments downwind of sewage treatment plants.

Experience gained from measuring fluorescence signals of single bacterial spores under flow cytometry using UV excitation at 340-360 nm, was applied to concept testing of a prototype instrument, built to do the same for aerosols. This machine was capable of resolving particle size as well as fluorescence intensity of each particle under laboratory and field conditions; it was called the fluorescent aerodynamic particle sizer (FLAPS). A second generation FLAPS (FLAPS2) was designed to be smaller, power efficient and field portable. FLAPS2 was challenged under refereed conditions in blind trials to determine if it could detect biological aerosols. This paper describes practical aspects of measuring biological aerosols when the results must be compared to reference samplers that provide culturable or "live" data. Treatment of particle size and fluorescence information is discussed with respect to FLAPS and reference data fidelity. Finally, an objective method is introduced to evaluate FLAPS data correlation to reference data. The results suggest that there is positive correlation between FLAPS measurements and live biological aerosol particles.

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